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**INDUCED LEVELS OF HEPATIC MIXED  
FUNCTION OXIDASES IN  
WHITE SUCKERS  
(*Catostomus commersoni*)  
AS A BIOMARKER FOR  
CONTAMINATION OF THE  
SEDIMENTS IN JACKFISH BAY,  
AND THE KAMINISTIGUIA AND  
ST. MARY'S RIVERS**

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## EXECUTIVE SUMMARY

Mixed function oxidases (MFOs) are a family of inducible enzymes which oxygenate natural and synthetic chemicals alike, making them amenable to conjugation and excretion. The levels of these enzymes are induced, particularly in the liver, in fish exposed to classes of chemicals including dioxins, furans, polynuclear aromatic hydrocarbons, polychlorinated biphenyls and other chemicals exhibiting "dioxin-like" activity. The induction of MFO's is the basis for the dioxin-equivalents applied to the individual congeners of dioxins, furans and polychlorinated biphenyls to determine their relative activity.

MFO induction in wild white suckers was investigated as a potential biomarker (indicator) for contamination of sediments by chemicals with dioxin-like activity in three International Joint Commission designated "areas of concern", Jackfish Bay, St. Mary's River and the Kaministiquia River.

MFO determinations in spawning suckers suggested no induction, although white suckers captured in the summer from Jackfish Bay had MFO levels up to 6 (2,5-diphenyloxazole metabolism) and 7 (Benzo-a-pyrene hydroxylase) fold higher than did reference suckers. Similar induction was found in suckers collected in the summer from the Kaministiquia River and from the St. Marys' River, hepatic MFO activities 3 (PPO) and 5 (BaP) times those of the reference populations suggesting exposure to chemicals with MFO inducing potential.

The induction of hepatic MFO's at all three polluted locations is a sensitive measure of industrially discharged xenobiotics which exhibit dioxin-like activity. The induction of MFO's in these wild fish populations also indicates that these discharged xenobiotics affect the health of these organisms.

MFO induction may be a useful biomarker for monitoring the efficiency of remedial action plans. MFO analysis may also supplement traditional chemical analysis for the range of compounds with dioxin-like activity as it automatically considers the antagonistic and synergistic effects of complex mixtures of these chemicals, and also provides information on only the biologically available component of these chemicals.



## INTRODUCTION

The very high levels of organic and inorganic contaminants (xenobiotics) found in the sediments of some harbours, bays and rivers around the Great Lakes has prompted the International Joint Commission to designate these as "areas of concern" (Great Lakes Water Quality Board 1982). The effects of these contaminants need to be investigated in a variety of trophic levels and species. Benthic species which reside in contaminated areas for extended periods of time are exposed to xenobiotics directly from the sediment, from the over-lying water and from their diet. The impacts of xenobiotics on such populations and communities may be seen as changes in abundance or structure (Johnson 1984, Klontz 1984). These changes likely develop from a variety of more subtle biochemical alterations which are very specific to a chemical's "mode of action" and which themselves are very sensitive indicators of xenobiotic exposure (Passino 1984). The white sucker, (*Catostomus commersoni*), a benthic fish species with relatively sedentary habits, has proven useful as a monitor for carcinogenic contamination of the sediments (Smith *et al* 1989, Smith and Rokosh 1989, Cairns and Fitzsimons 1988, Hayes *et al* 1990) and for biochemical investigations (Kirby *et al* 1989, 1990, Munkittrik *et al* 1989). Biochemical alterations in benthic organisms, such as white suckers, may be a very sensitive indication of chemical effects in areas of concern.

Mixed function oxidases (MFOs) are a family of enzymes which have been conserved through evolution and which are abundant throughout the animal (vertebrate) kingdom (Payne 1984). The normal role of some MFO's in the homeostasis of organisms may be to regulate steroid hormone levels (Snowberger and Stegeman 1987, Kleinow *et al* 1987, Stegeman and Kloepper-Sams 1987). Other MFO isoenzymes are able to oxygenate foreign (xenobiotic) chemicals, making them amenable to conjugation and excretion (see reviews by Buhler and Williams 1989, Stegeman and Kloepper-Sams 1987, Kleinow *et al* 1987), but the normal function of these isoenzymes has not been determined. Many chemicals, including poly-chlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins and dibenzo-furans, and polynuclear aromatic hydrocarbons (PAHs) are potent inducers of these latter iso-enzymes (see reviews above). MFO induction is modulated by the affinity of these chemicals for the cytosolic (Ah) receptor which acts as an intermediary step in the induction of MFO's (Poland *et al* 1976). The induction of MFOs, in association with such contaminants, has been documented in several fish

populations in the Great Lakes (Binder and Lech 1984, Luxon *et al.* 1987, Fabacher and Baumann 1985). The induction of MFOs is a significant biological effect that is specific for chemicals with this mode of action, binding to the Ah receptor, with a range of consequences. The induction of hepatic MFOs may potentiate the toxicity of other chemicals, including carcinogens (Bailey *et al.* 1987, Kleinow *et al.* 1987, Stegeman and Kloepper-Sams 1987, Buhler and Williams 1989). In Puget Sound the susceptibility of various species to develop cancer may be related to their MFO levels (Collier and Varanasi 1988). Induced MFOs are also associated with reductions in reproduction (Spies *et al.* 1985, 1988, Spies and Rice 1988, Johnson *et al.* 1988, Munkittrik *et al.* 1989).

The levels of MFOs in the liver of white suckers (*Catostomus commersoni*) captured in the Kaministiquia River (Thunder Bay), Jackfish Bay (Terrace Bay) and the Saint Mary's River (Sault Ste. Marie) were found to be elevated when compared to levels in suckers from reference (uncontaminated) populations. The levels of an enzyme responsible for the conjugation of the products of MFO action, UDP-Glucuronic acid transferase (UDPGT), were depressed at two of these locations. These results suggest that, for three areas of concern on the uppermost of the Great Lakes, sediment contamination is sufficient to have a deleterious impact on the sub-cellular biochemistry of these fish.



## METHODS

### Study Locations:

White suckers were captured with trap-nets in September 1987 from the Saint Mary's River (n=10; study sites are illustrated in Figure 1) immediately below the power dam, and compared with suckers (n=34) from Batchewana Bay (reference site) captured in 4.5 inch stretch gill nets. In 1988 white suckers (n=20) were captured from the spawning migrations in Sawmill Creek, which discharges into Jackfish Bay (impacted), and from the Little Gravel River (n=20), which discharges into Mountain Bay (reference site). These fish were captured with a 4 foot hoop net placed in the mouth of the spawning streams. In the summer (August) of 1988 white suckers were captured from a variety of areas using 4 inch gill netting. Jackfish Bay (n=26) was sampled with gill nets placed immediately below the plume discharged by a bleached kraft pulp and paper mill, while in Mountain Bay (n=25) the nets were placed in 15-25 feet of water approximately 0.5 miles off the mouth of the Little Gravel River. Suckers were also captured from Black Bay (n=34), which served as a reference site for fish taken in the Kaministiquia River (n=30), immediately below the turning basin, and for fish taken from Mission Bay (n=34), where the Kaministiquia River discharges into Lake Superior.

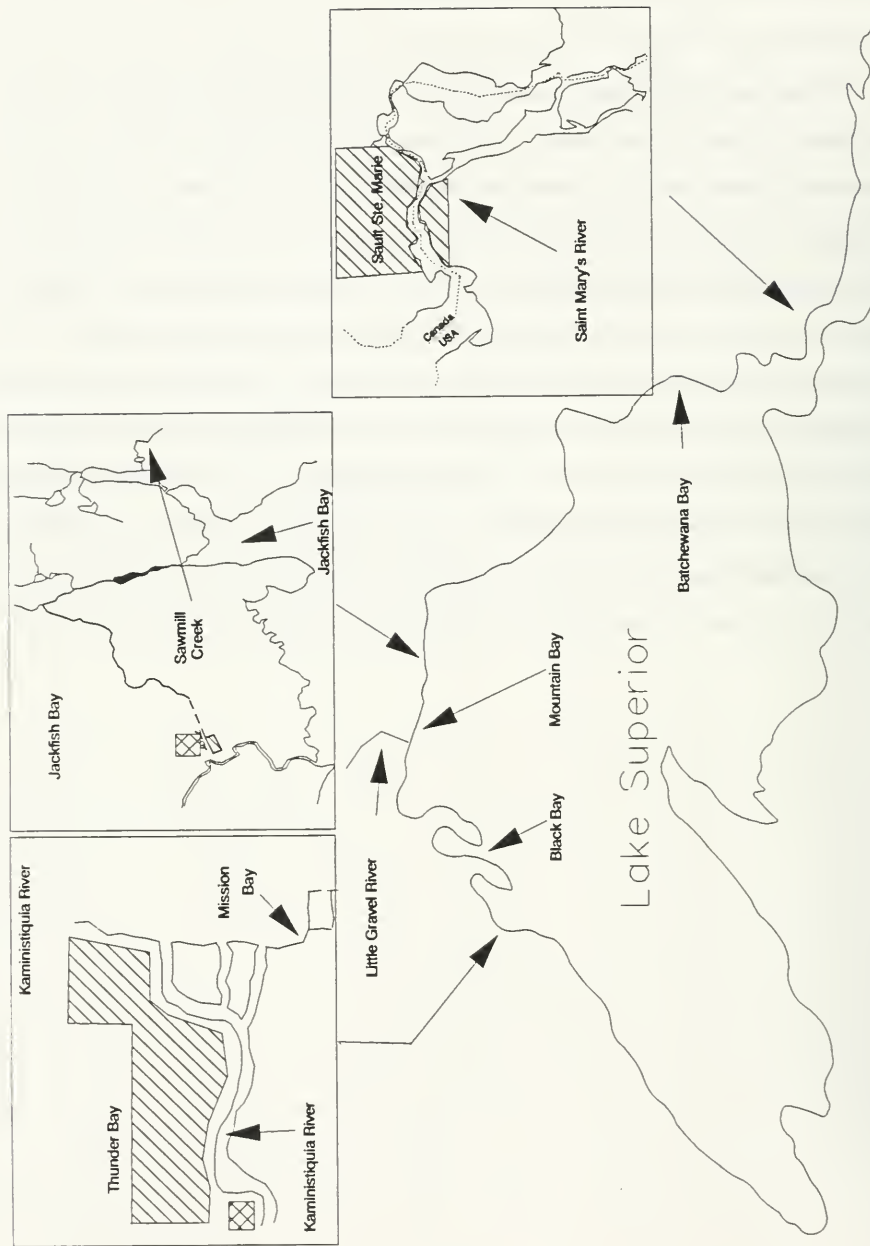


Figure 1: Study locations in Lake Superior and the Saint Mary's River.

#### MFO analysis:

Fish taken alive were killed with a blow to the head, weighed, sexed, length determined, and a full autopsy performed. Liver tissue was excised, rinsed with cold 1.0% KCl, minced and frozen in liquid N<sub>2</sub>. In 1987 samples for 2,5-diphenyloxazole determinations were thawed on ice, homogenized with a motorized teflon mortar and pestle with 2 volumes (V/W) homogenizing buffer, 50 mM Tris, pH 7.5, with 3.0 mM MgCl<sub>2</sub> and 0.2 M KCl, after Luxon *et al.* (1987). In 1988 livers were homogenized in 3 volumes of buffer (0.1 M KPO<sub>4</sub> buffer, pH 7.4, 1 mM dithiothreitol, 1 mM EDTA, 20 % glycerol). Supernatant after 9000 XG centrifugation for 20 min. (S9) was used fresh in subsequent analysis for mixed function oxidase analysis with 2,5-diphenyloxazole (PPO) as the substrate after Luxon *et al.* (1987). Protein was determined by the Bradford binding assay (Bradford 1976) with bovine serum albumin as the standard. The supernatant (S9) was subsequently ultracentrifuged for 1 hour at 40,000 rpm (100,000XG) to sediment the microsomes, which were resuspended in 1 mL buffer for every gram original wet weight, yielding 15-25 mg. mL<sup>-1</sup> protein. Mixed function oxidase activity in microsomes was determined with 1.0 mg. protein mL<sup>-1</sup> reaction mixture with benzo(a)pyrene as the substrate, after Nebert and Gelboin (1968). The activity of benzo-a-pyrene hydroxylase was converted from fluorescent units min<sup>-1</sup> mg protein<sup>-1</sup> to nM BaP hydroxylated using 3-hydroxy benzo-a-pyrene (1 FU equals 12.1 nM BaP-OH in the reaction volume) as a standard. For 2,5-diphenyloxazole the fluorescence of a quinine sulphate standard (1 FU equals 0.41 µg quinine sulphate L<sup>-1</sup> 0.5 N NaOH) was used to calibrate the equipment, however because the product of the 2,5-diphenyloxazole is unavailable for standards, this data is presented as fluorescent units.

Uridine-5'-diphosphoglucuronic acid transferase (UDPGT) activity was determined in microsomes after Castren and Oikari (1983) with 5 mg. protein in a reaction mixture of 1 mL, the activity being expressed as nM p-nitrophenol conjugated min<sup>-1</sup> mg protein<sup>-1</sup>.

Data was analyzed for homogeneity of variance, which was lacking, so it was subsequently analyzed by Tukey's multiple range test after log<sub>10</sub> transformation, which produced homogeneous variances.

## RESULTS

In many comparisons of MFO activity significant differences between sexes were identified, so in all instances the sexes are considered separately. The effect of season on the induction of mixed function oxidases in the Jackfish Bay area is shown in Figure 2. Males had significantly higher activity in the spring than did females, but no differences between locations was evident. In the summer the sexes were similar, but the activity in Jackfish Bay suckers was 6 and 7 times reference values for males and females respectively, suggesting the Jackfish Bay suckers are not exposed to MFO inducers in the spring, or possibly are not inducible in the spring.

Mixed function oxidase activity with PPO as the substrate was determined at all sites in summer caught fish and is shown in Figure 3. Sex differences were evident in some locations in the summer, males having higher activity in 7 of 8 comparisons but unlike the spring samples none of these differences were statistically significant when the entire data-set (16 cases) was tested (Tukeys,  $P > 0.05$ ). Subsequent analysis for males or females separately showed significant induction in Kaministiquia River females relative to both Black Bay and Mission Bay females. The intermediate activity evident in males from Mission Bay at the mouth of the Kaministiquia suggests some induction at this location also, but neither this nor the activity in Kaministiquia River males was different from reference (Black Bay) values. Both males and females from Jackfish Bay were induced when compared to Mountain Bay suckers. The PPO activity in males and females from Batchewana Bay was significantly lower than in suckers from the Saint Mary's River. The PPO activity in male suckers taken from the outer reaches of Batchewana Bay was somewhat higher than expected (Figure 3), but this was not significant. These two samples (inner and outer) could have been combined, but it is interesting to note that this elevation in males from the Outer bay is marked when compared to the other populations at Black Bay and Mountain Bay.

Hepatic MFO activity with BaP as the substrate was only determined at the 1988 sampling locations, and is summarized in Figure 4. Differences between the sexes were not as prevalent as with PPO as the substrate, males being higher in only 2 or 5 cases, none of these differences being statistically significant. In the Kaministiquia River both males and females were induced relative to Black Bay, as were females from Mission Bay. Both males and females in Jackfish Bay were induced relative to Mountain Bay reference fish.

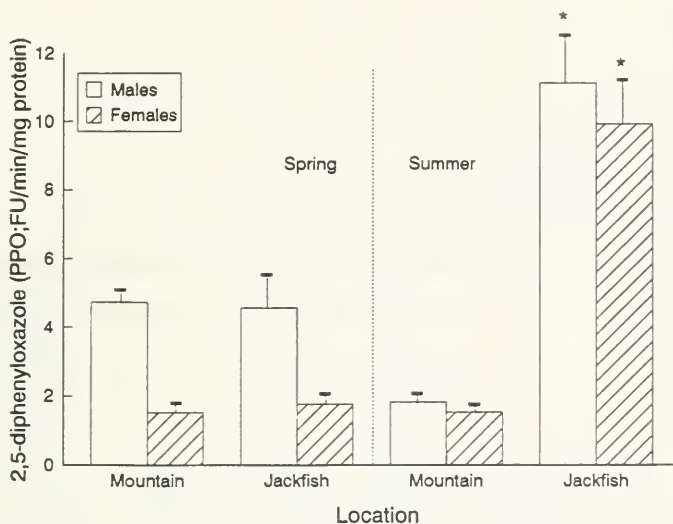


Figure 2: The activity (mean, S.E.) of hepatic mixed function oxidase activity with 2,5-diphenyloxazole as the substrate in spring and summer samples of white suckers from Jackfish Bay (contaminated) and Mountain Bay (reference). Significant induction compared to the same sex from the reference site is indicated (\*).

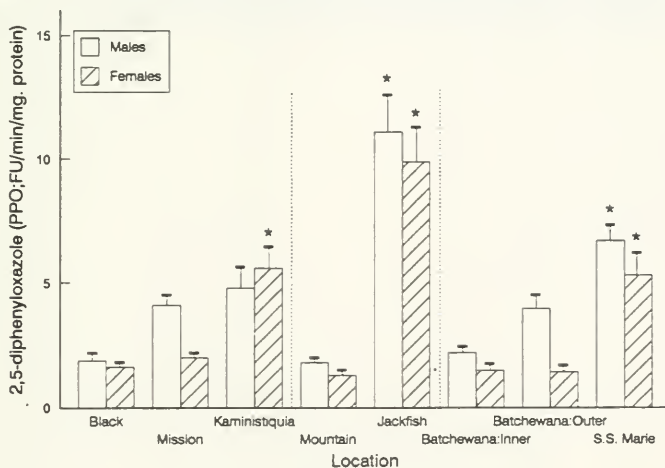


Figure 3: The activity (mean, S.E.) of hepatic mixed function oxidases measured with 2,5-diphenyloxazole as the substrate in summer caught white suckers from all study sites. Significant induction in test sites compared to the same sex from the reference sites, each grouping (test and reference) separated by a dashed line, is indicated (\*).

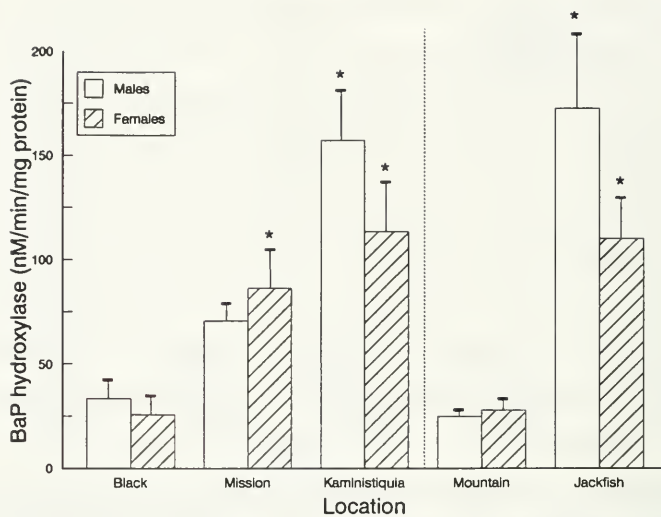


Figure 4: The activity (mean, S.E.) of hepatic mixed function oxidases measured with benzo(a)pyrene as the substrate in white suckers caught during the summer of 1988, each grouping (test and reference) separated by a dashed line. Significant induction relative to reference fish (same sex) is note (\*).

While the same fish were not consistently analyzed for both BaP-hydroxylase and 2,5-diphenyloxazole activity, a good correlation ( $r^2=0.64$ ) in mean values from each site for these two enzymes is evident, shown in Figure 5. The levels of hepatic MFOs measured using both substrates suggests induction of these enzymes at all three areas of concern.

Microsomal UDPGT activity (Figure 6) was slightly lower in suckers from all three sites (Mission, Jackfish and Kaministiquia) impacted by pulp and paper discharges, compared to reference locations (Black, Mountain), however these differences were not significant statistically.

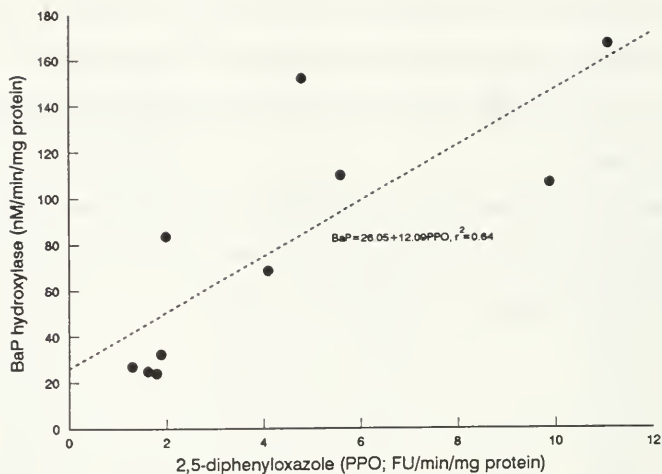


Figure 5: Correlation between the average (sexes separate) activities of hepatic 2,5-diphenyloxazole and benzo(a)pyrene metabolism in the various locations sampled in 1988.

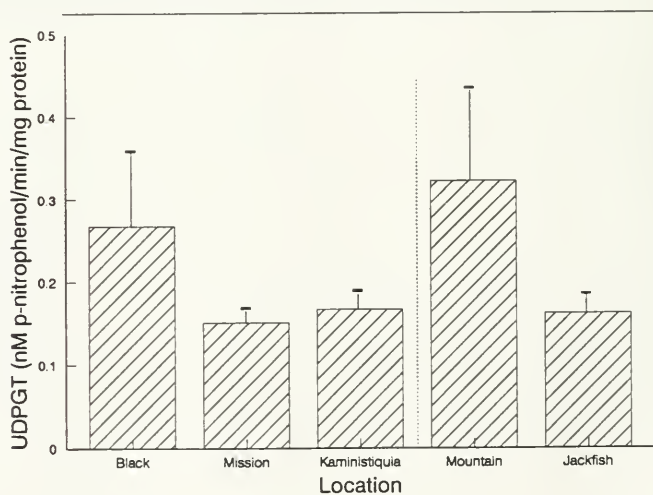


Figure 6: Average microsomal activities (S.E.) of UDP glucuronic acid transferase in liver preparations from white suckers sampled in 1988. Each grouping of test and reference locations is separated by a dashed line.



## DISCUSSION

The induction of MFOs in benthic species (white suckers) caught in "areas of concern" such as the Kaministiquia River, Jackfish Bay or the St. Mary's River, likely reflects localized contamination of the sediments, water and benthic invertebrates. In contrast the induction of MFOs in pelagic species such as Lake Trout (Luxon *et al.* 1987, Binder and Lech 1984, Hodson *et al.* 1989) correlates with and implicates chemical inducers which bioaccumulate in the Great Lakes pelagic food-chain, such as PCBs, dioxins or furans. Several factors suggest that PCBs are not responsible for the MFO induction seen in benthic white suckers in the present study. Firstly, PCB levels, which were less than 80  $\mu\text{g g}^{-1}$  in the muscle of white suckers from all of the sites in the present study (C. Cox, Environment Ontario, 4th Floor, 1 St. Clair Ave. W., Toronto, Ont., pers. communication) do not exceed the threshold for MFO induction (300  $\text{ng. g}^{-1}$ ) suggested by Melancon *et al.* (1989), but induction is present never-the-less. Secondly, the range of PCB levels in suckers (from non-detectable to a maximum of 80  $\mu\text{g g}^{-1}$  in the Kaministiquia River area) do not correlate well with the 7 fold induction in hepatic MFO levels (not shown). Thirdly, the levels of hepatic MFOs in white suckers from Jackfish Bay, Kaministiquia River and the St. Marys River are similar to MFO levels in white suckers from western Lake Ontario, with 800  $\mu\text{g g}^{-1}$  PCB in their muscle (I. Smith, unpubl. observations), despite the 10 fold difference in PCB levels. These differences suggest that benthic species such as white suckers also must respond to contaminants which do not bioaccumulate in the food chain, such as polynuclear aromatic hydrocarbons and resin acids (Fabacher and Baumann 1985, Van Veld *et al.* 1988, 1990, Mather-Mihaich and Di. Giulio 1989) to which pelagic species such as lake trout are not exposed. Point-source discharges of chemicals such as PCB's, dioxins and furans may lead to localized contamination of the sediments (Sherman *et al.* 1989), with little accumulation in the pelagic food chain, but with a likely impact on the benthic food chain. The level of hepatic MFO's in lake trout may be appropriate for monitoring remediation at the "whole lake" level (Ryder and Edwards 1985) such as suggested by Marshall *et al.* (1987) in the dichotomous key for lake trout as an indicator of ecosystem health. The levels of hepatic MFOs in white suckers or brown bullheads may be more useful for localized problems, such as in areas of concern.

The induction of hepatic MFOs observed in white suckers from these three "areas of concern" suggests significant exposure to chemicals with MFO inducing properties. The white sucker has proven useful for

epidemiological studies in part because of its tendency to home to and spawn in small streams in the spring, when large numbers of fish are easily sampled. Because the activity of MFOs varies with season (witness the lack of induction in the Jackfish Bay population during the spawning migration) summer samples may be more appropriate for MFO analysis. Luxon *et al.* (1987) have also noted seasonal difference in hepatic MFOs of lake trout from western Lake Ontario suggestive of an effect of spawning on MFO induction. Clearly spawning inhibits (Lindstrom-Seppa 1985) but does not prevent the induction of MFOs (Walton *et al.* 1978, 1983, Spies *et al.* 1985, 1988, Collier *et al.* 1986, Kleinow *et al.* 1987). At other Great Lakes locations induction has been noted in spawning populations of white suckers but not in the same population during their summer lake-resident period. This anomaly was attributed to contamination of the estuary of the spawning stream but not of the lake sediments (I. Smith, unpubl. data). A variety of hepatic MFO isoenzymes are inducible in fish (see Buhler and Williams 1989, Stegeman and Kloepper-Sams 1987, Kleinow *et al.* 1987) which vary in their catalytic abilities, substrates and structures. Weak MFO induction might disappear during spawning because more energy is put into the steroid metabolizing forms (not detectable with BaP or PPO) than into xenobiotic metabolizing isoenzymes. It is also possible that in migrating to the spawning stream, 1.5 km from the warm-water discharge by a pulp and paper mill into Jackfish Bay, exposure to inducing chemicals is reduced, and MFOs decrease. Field collections for the quantification of hepatic MFO levels must address this issue in detail, balancing the ease and inexpense of spring captured fish versus the clear induction in summer fish, which are more difficult (expensive) to obtain. In either case the use of either BaP (microsomes) or PPO (homogenate) appears to be equally suitable for measuring the induction of these xenobiotic inducible isoenzymes.

Two of the three sites in which sucker MFOs were induced receive discharges from bleached kraft pulp and paper mills, though in the Kaministiquia River municipal sewage, coal dust, grain waste and other industries also impact on the estuary. The sediments and water of the lower Kaministiquia River are contaminated with oils and grease, heavy metals, PCBs, resin acids, chlorophenols and PAHs (Jaagumagi 1990). Although the PCBs contaminate the sediments, levels in white suckers ( $80 \text{ ug g}^{-1}$ ) are not remarkably elevated compared to reference (less than the detection limit of  $20 \text{ ug g}^{-1}$ ; C. Cox, Environment Ontario, 4th Floor, 1 St. Clair Ave. W., Toronto, Ont., pers. communication). The induction of hepatic MFOs has been reported in fish caged in the effluent of

pulp mills (Lindstrom-Seppa and Oikari 1989), caught from within the impact zone (Andersson *et al.* 1988, Larsson *et al.* 1988, Rogers *et al.* 1989), or exposed in the laboratory (Andersson *et al.* 1987). Resin acids, which are peculiar to pulp and paper mills, have recently been shown to induce hepatic MFOs (Mather-Mihaich and DI Gulio 1989), and dibenzo-p-dioxins and furans (potent inducers) have been identified in pulp mill sludge and discharges (Clement *et al.* 1987, Mah 1989) and more importantly in white suckers from Jackfish Bay (Sherman *et al.* 1989). The intermediate response of MFOs in suckers taken from Mission Bay at the mouth of the Kaministiquia River indicates that 1), the zone of impact extends into Lake Superior itself, or 2), induced suckers may have been exposed in the river but were captured in Mission Bay several km. downriver, or 3) the spoils from dredging of the river dumped in Mission bay are contaminated with MFO inducing xenobiotics also. Thus it seems likely that the MFO induction in both populations is due at least in part to the discharges from the pulp and paper mills.

The reduction in hepatic microsomal UDPGT noted in the Kaministiquia River, Mission Bay and Jackfish Bay suckers is also consistent with the reported impacts of pulp mills using chlorine bleaching (Oikari *et al.* 1984, 1985). Changes in MFO and UDPGT levels are important because UDPGT acts to conjugate (detoxify) many of the compounds metabolized by the MFOs, such as chlorinated phenols and resin acids (Oikari and Anas 1985, Mattsoff and Oikari 1987). Because UDPGT is rarely induced in concert with MFO induction (see Foureman 1989) an imbalance between oxidation (MFO's) and conjugation (UDPGT) could lead to increased effects of chemicals, such as PAH's, which are made more toxic by MFO's. Depression of UDPGT may lead to the jaundice noted in fish exposed to pulp mill wastes (Mattsoff and Oikari 1987) by a failure to conjugate bilirubin, although jaundice could also partially arise from resin acid effects on red blood cells (Busshell *et al.* 1985).

The induced levels of MFOs noted in the white suckers taken below the rapids in S.S. Marie correlate with the contamination of the sediments in this area by a wide range of chemicals including PCB's, PAH's and heavy metals (Shimizu and Finch 1988). The primary impacts in this area are a large steel smelter and a municipal sewage outfall. The induction of MFOs has also been noted in the Black River in Ohio, in Hamilton Harbour in Ontario, in Puget Sound in Oregon and in the Elizabeth River in Virginia, all sites receiving PAH's

from industrial sources (Fabacher and Baumann 1985, Collier *et al.* 1986, Hodson *et al.* 1989, Van Veld *et al.* 1990). The induction of MFOs by PAH's is important (in contrast to induction by PCB's) in light of the carcinogenicity and mutagenicity of PAH's, such as benzo-a-pyrene, after oxygenation to a reactive intermediate by MFOs. The unusual MFO levels in male suckers taken from the outer portion of Batchewana Bay, approaching those found in the Kaministiquia River and Mission Bay, cannot be explained by PCB (less than the 20 ug g<sup>-1</sup> detection limit) or other (DDT,DDE, chlordane, mirex) contamination. Hodson *et al.* (1989) also noted anomalous MFO levels in fish from eastern Lake Superior. The anomalous findings in eastern Lake Superior do not however detract from the likelihood that the induced levels of hepatic MFOs in the St. Marys River suckers may be due to the PAH contamination of the sediments.

The consequences of MFO induction in these fish populations has not been clearly identified, but several studies implicate xenobiotics (such as those metabolized by MFOs) in health defects. An abnormal incidence of liver neoplasms (cancers) has been identified in white suckers from the three areas of concern in the present study (Smith and Rokosh 1989, Smith unpubl. data) and also in brown bullheads from Munuscong Bay, adjacent to the Saint Mary's River (IJC 1987). The induction of MFOs in Jackfish Bay also correlates with disruptions of the reproductive process and steroids (Munkittrik *et al.* 1989). The use of biochemical changes (MFOs) specific for chemicals (dioxins, furans, PCBs, PAH's) with defined modes of action (Ah binding) may be sensitive measures of xenobiotic impacts suitable for monitoring the progress of remediation measures in these and other areas of concern. If reductions in biochemical indicators (such as MFOs) are noted after remediation, additional studies on fish health and population structures may be warranted.

## CONCLUSIONS

1. Contamination of the sediments in three Northern Ontario "Areas of Concern" is sufficient to induce biochemical effects in resident populations of white suckers.
2. These induced biochemical changes suggest the mixtures of contaminants present in these areas are "dioxin-like" in character and activity.
3. The identity of specific chemicals was not investigated, however it is likely that dioxins, furans and resin acids (discharged by bleached kraft mills) which contaminate the Kaministiquia River and Jackfish Bay, and polynuclear aromatic hydrocarbons which contaminate the St. Mary's River, are at least partly responsible.
4. The inhibition of Uridine 5'-diphosphoglucuronic acid transferase in the Kaministiquia River and Jackfish Bay sites is also likely due to the discharges by the bleached kraft mills impacting on these sites.



## RECOMMENDATIONS

1. Biochemical monitoring of the white sucker populations (MFO and UDPGT) be carried out to determine the effectiveness of remedial and other (institution of secondary treatment by bleached kraft mills) measures undertaken in these sites to reduce the levels of sediment and other contamination.
2. Biochemical monitoring be incorporated into biomonitoring programs which currently utilize costly and time-consuming analytical techniques to determine PCB, dioxin, furan and polynuclear aromatic hydrocarbon levels in vertebrate biota.
3. Additional "biomarkers" for contaminant effects be investigated for incorporation into biomonitoring schemes.
4. The induction of MFO's be investigated as a routine testing methodology to complement the GC/MS/MS analysis of environmental samples, including sediments and biota. This type of analysis automatically incorporates mixture effects (synergism, antagonism) which cannot be determined using the present congener specific analysis combined with toxic equivalency factors.





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